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Plasmid Association and Nucleotide Sequence Relationships of Two Genes Encoding Heat-Stable Enterotoxin Production in *Escherichia coli* H-10407

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Plasmid DNA from enterotoxigenic *Escherichia coli* strains H-10407 and H-10407-P was examined for nucleotide sequence homology to two *E. coli* genes encoding infant mouse-active heat-stable enterotoxins (ST). A 62-megadalton plasmid of strain H-10407 contained sequences homologous to the gene encoding a toxin designated STIb, previously isolated from a human isolate of *E. coli*. A 42-megadalton plasmid of strains H-10407 and H-10407-P contained sequences homologous to the gene encoding a toxin designated STIa, previously isolated from bovine and porcine isolates of *E. coli*.

Molecular cloning, nucleotide sequence analysis, and nucleic acid hybridization studies have demonstrated at least two related but heterogeneous genes encoding infant mouse-active heat-stable enterotoxin (ST) production among enterotoxigenic *Escherichia coli* (ETEC) isolated from humans (6, 7, 9). One of these genes encoding a toxin designated STIa was isolated from a bovine ETEC isolate and was characterized by So and McCarthy (10). Hybridization studies have revealed nucleotide sequences with a high degree of homology to the STIa gene among ST-producing ETEC isolated from calves, pigs, and humans (6, 7, 8). The second gene, which encodes a toxin designated STIb, was isolated from a human ETEC isolate and shows a significant degree of nucleotide sequence divergence from the STIa gene (7). Preliminary studies suggest that the STIb gene is limited to human ETEC isolates (S. L. Moseley and H. W. Moon, unpublished data).

Studies with isolated fragments of DNA from these genes as hybridization probes have suggested that some human isolates of ETEC may possess both of these genes (6, 7). Yamamoto and Yokota (13) have recently shown that *E. coli* H-10407, an extensively studied isolate from a person with diarrhea in Bangladesh, has two plasmids which encode ST. These authors also discussed serological evidence suggesting that the toxins encoded by these two plasmids are heterogeneous. In the present study, we demonstrate that there are two genes encoding ST production in strain H-10407. We show that a 42-megadalton (Mdal) plasmid of strain H-10407 encodes STIa, and a 62-Mdal plasmid encodes STIb. We further show that the derivative strain H-10407-P, which has spontaneously lost the 62-Mdal plasmid and the ability to produce ST (1, 3), retains sequence homology with the STIa gene.

Agarose gel electrophoresis of plasmid DNA purified from strain H-10407 (Fig. 1A) reveals the presence of two plasmid classes of 62- and 42-Mdal as reported by others (2, 11, 13). A 3.7-Mdal plasmid of strain H-10407 was allowed to run off the gel to improve the resolution of the larger plasmids. The 42-Mdal class was recently shown to consist of two distinct plasmid species of identical molecular sizes (13). Strain H-10407-P (Fig. 1A) retains at least one of the 42-Mdal plasmids, but has lost the 62-Mdal plasmid as previously reported (3). Plasmid DNA from this gel was transferred to diazophenylthioether cellulose (DPT; Schleicher and Schuell, Keene, N.H.) and probed with radiolabeled DNA from the ST-encoding region of plasmid pRIT10036, a recombinant plasmid carrying a gene from a porcine ETEC isolate homologous to the STIa gene (5, 6). The probe hybridized to the 42-Mdal plasmid DNA of H-10407 and H-10407-P (Fig. 1B). So et al. (8) have also reported that sequences homologous to the STIa gene are present in H-10407-P, but the strain does not produce ST detectable in the infant mouse assay (1, 2) or in ligated intestinal loops of neonatal piglets (S. C. Whipp and S. L. Moseley, unpublished data). Yamamoto and Yokota (12) demonstrated that the gene is functional in other host strains

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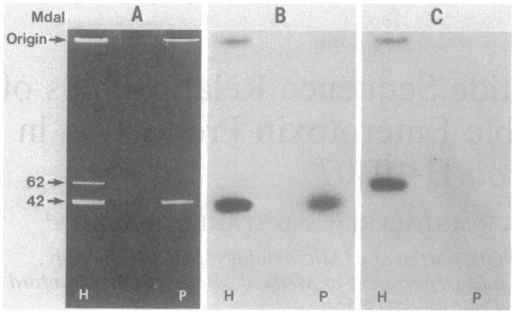

FIG. 1. Agarose gel electrophoresis and DNA hybridization of plasmid DNA from strains H-10407 and H-10407-P. Plasmid DNA was isolated as described by Kado and Liu (4) and electrophoresed in 0.7% agarose. Transfer of DNA from the agarose gel to DPT paper and subsequent hybridization at high stringency, washings, and rehybridization were performed by methods provided by the manufacturer (Schleicher and Schuell technical publication no. 352-4). Preparation of ^{32}P-labeled probe DNA was as previously described (6, 7). (A) Agarose gel electrophoresis of plasmid DNA. (B) Hybridization of DPT blot of gel shown in (A) to STIa gene probe. (C) Hybridization of DPT blot of gel shown in (A) with STIb gene probe. (H) H-10407; (P) H-10407-P.

since conjugal transfer of the 42-Mdal plasmid from strain H-10407 could confer upon a nontoxigenic recipient strain the ability to produce detectable ST. They also showed that a recombinant plasmid carrying the ST gene from the 42-Mdal plasmid of strain H-10407 could confer upon transformants the ability to produce detectable ST. The basis of the failure of strain H-10407-P to produce detectable levels of ST is not understood.

The STIa gene probe was removed by washing the DPT paper, and the paper was then probed with a fragment of DNA from the STIb-encoding region of the recombinant plasmid pSLM004 (7) (Fig. 1C). The 62-Mdal plasmid from strain H-10407 was detected by the probe, whereas no homologous sequences were detected in plasmid DNA from strain H-10407-P. These observations confirm that strain H-10407 possesses two heterologous ST genes showing homology to the genes encoding STIa and STIb, and allows assignment of the STIa gene to the 42-Mdal plasmid, and the STIb gene to the 62-Mdal plasmid. The failure of strain H-10407-P to produce detectable levels of ST suggests that the toxin produced by strain H-10407 is STIb. Although this possibility might raise a question of the significance of STIa in human disease, previous hybridization studies have shown that DNA from several human isolates of ETEC which produce only ST show homology exclusively to the STIa gene (6, 7).

Yamamoto and Yokota (13) report preliminary evidence based on restriction endonuclease analysis which suggests that neither ST gene in H-10407 is part of Tn*1681*, a bacterial transposon carrying the STIa gene reported by So et al. (9). In the present study, we used probes consisting primarily of the ST-encoding sequences of the STIa and STIb genes, and thus do not address the question of sequences neighboring the ST genes which may constitute transposons. It is clear from our data, however, that the ST-encoding sequence of the 42-Mdal plasmid of strain H-10407 is highly homologous with the STIa gene described by So and McCarthy (10). The possibility that the gene may exist as Tn*1681* in some strains while not in others raises interesting questions concerning the molecular epidemiology and evolution of ST genes in *E. coli*, as does the presence of two heterologous genes in the same strain, each encoding a product with similar, if not identical, biological activities.

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